DePamphilis, Melvin, Arup Chakraborty, and Matthew Frieman 2021

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Behind the Mask

March 9, 2021

GB: Good afternoon. Today is March 9, 2021, and I have the pleasure of speaking to Dr. Melvin DePamphilis, Dr. Arup Chakraborty, and Dr. Matthew Frieman. Dr. DePamphilis and Dr. Chakraborty both are in the Section of Eukaryotic DNA Replication at the Eunice Kennedy Shriver National Institute of Childhood Health and Human Development, and Dr. Frieman is an associate professor of microbiology and immunology at the University of Maryland School of Medicine. Thank you all for being with me today. Thank you.

My first question is, can you explain in lay terms how lysosome homeostasis affects the cell's response to COVID-19? My understanding is that is the premise of your COVID research right now.

MDe: I would like Matt to address the questions about the virus because he is actually an internationally recognized authority on these RNA envelope viruses, don't you agree, Matt? But it's easier it seems if we just take turns and comment where we feel we have something to say.

MF: Well, why don't you start with the lysosomal side and what that role is during kind of general cell biology, and then I'll jump in with the virus side.

MDe: Okay, Gabrielle, I sent you a picture of the diagram basically and I sent Matt. You have a copy and Arup has a copy. It's a simple diagram which points out that the virus has to enter the cell, and it needs at least two fundamental mechanisms. Fundamental one: it has to have a specific cell surface receptor. In this case it's called ACE2. It has nothing to do with biology and everything to do with angiotensin, but the virus has evolved so that it recognizes this protein. It's not found in mice; it is found in humans, and if you have this protein, you're likely to be sensitive to getting infected by a virus such as SARS-CoV-2 that recognizes it, as it has to bind to the membrane.

The second thing is: it has to be encapsulated and brought into the cell by a process people generally call endocytosis. [There's] a number of different names for it, but it means the membrane folds around the virus, puts it into a little spherical ball, brings it into the cell, and then this spherical ball, as I tried to indicate in the diagram, has stages. There are early endosomes, late endosomes. They go through stages. There are biochemical changes in the enzymes that are associated with membranes and so forth and there are data in the literature that strongly suggests that the enzyme called PIKFYVE, just a fancy name for the enzyme, is essential for this process of endosome maturation. Therefore, if the virus has to go through this process and we inhibit the enzyme which is required for that process to function correctly and bring the virus into the cell, then the virus literally becomes trapped in there.

Now what would happen next, I don't know. Matt pointed out to me something I hadn't appreciated earlier, in that there are also these double membrane vesicles. It seems quite clear now that these double membrane vesicles are where the virus actually replicates its proteins in its RNA and makes new viruses. They exit the cell pretty much the way they came in through exosomes, again encapsulation membrane, it buds off, and out comes the virus.

So here's the bottom line. These enzymes, a series of them and PIKFYVE, are critical for all of this protein trafficking that goes on in the cell, and for endosomes and exosomes and autophagosomes and auto endolysins and all kinds of things are members of this trafficking mechanism bringing proteins into the cell, bringing proteins out of the cell, encapsulating the bacteria, encapsulating viruses, and in and out and so forth. So if we can inhibit a protein essential for bringing the virus into the cell, we've done something that nobody else would have been able to do before.

We are not targeting the virus. We are targeting a mechanism in the cell that is essential for the virus to survive. This is an important distinction and, Matt, you jump in when I make a mistake here. One of the things that intrigues me is we're developing a medication, not a vaccine. A vaccine goes after the virus. We go after the cell which the virus uses to propagate itself. So we could use these two forms—vaccines and medications together—but the interesting part is that, I think, Matt's done a lot of the thinking on this. It strikes me that this medication we're talking about, this ability to inhibit PIKFYVE, could be used probably for a great many of these RNA envelope viruses. Flu is one of them, for example, and other kinds of coronaviruses, too. I think it's going to have broad applications.

Now we can talk more about exactly how these inhibitors work, but if you want to focus on the virus, the critical question is can these types of inhibitors—they are inhibitors targeting a specific enzyme called PIKFYVE—can they prevent the infection of the cells in culture? Matt has beautiful data that says "Yes" and those data in fact have been offered as a patent application by the National Institutes of Health showing their support. Yes, so we're moving along here and basically the question then is, does it work in mice? Now Rup and I are not allowed to work with SARS in our building or in our animal facility, and Matt is one of the brave individuals who does work with the actual virus that is the cause of COVID-19. That is in the process. He and his students are in the process of determining whether what he has observed in cell culture is reproducible in mice. Matt, do you want to tell them more about how the mouse experiments work?

GB: I had a quick question just based on what you said. Are you all trying to inhibit PIKFYVE in the early stages when it enters the cell or at the later stages when they just want to exit the cell?

MF: Excellent question. Arup, do you want to address that?

AC: So, like Mel was trying to describe how the viruses get incorporated inside the cell, there are two mechanisms that we know about the SARS virus one. It is called endocytosis, by directly binding on the cell surface receptor and then just the genomic particle goes inside the cell. It also is known as receptor-mediated endocytosis. So PIKFYVE is the enzyme that is responsible for the maturation of the endosome from early endosome to late endosome. We are trying to inhibit the virus when it is entering via endocytosis. As soon as virus enters the cell and is encapsulated in that early endosomal vesicle, it is now getting transported by a network and ultimately it will go inside the cell and will get released inside the cytoplasm of the cell. During that process the endosomal vesicle gets mature from early endosome to late endosome and PIKFYVE is the only enzyme that is so far known responsible for the maturation of these early to late endosomes. Maturation of these endosomal vesicles are one of the important factors that help the release of the virus inside the cytoplasm where they can further replicate and spread the infection. By inhibiting PIKFYVE we are hoping that we are preventing the release of the viral particles inside the cell.

GB: Thank you and I think Dr. Frieman was going to talk about the mouse models.

MF: Just from the cell biology side, the other thing to remember is that the virus can enter cells two ways. It can enter through the endosome like Melvin described. It can also be cleaved at the spike protein on the outside of the virus of the membrane and then you can get fusion at the membrane and then release of the genome inside the cell. Different cells have different ways the virus has of getting in, depending on the cell. Each one of these is a major pathway. I think what we're certainly finding is that the PIKFYVE is functional at multiple aspects of this replication cycle, both as we said at the entry mechanism through the endosomes but also probably at these double membrane vesicles, DMV structures, that the virus induces during replication. By altering the lipid and membrane structure of the cell, we certainly are impacting replication of the virus. That's essentially what we're trying to work on with the project: to figure out where in the lifecycle of the virus this is working both in the cells and then also in the animal studies as well.

GB: This might be a little technical but how are you altering the lipid membrane structure? Is it like you're messing with the pH or is there some other thing that you're doing to alter that?

MF: I guess we're not altering the lipid stores in that sense like a cholesterol-inhibiting drug would do, but we certainly are going to affect the way that these vesicles fuse to each other and mature, and so there certainly are aspects of the virus replication that need these mature endosomes and lysosomes for their function whether it's entry of the virus or other aspects of replication cycle. I think that both of those may be playing a role in inhibiting the virus replication so that's what we're trying to figure out.

MDE: Along the same line you asked specifically about pH, which I found interesting. You probably are aware that lysosomes require an acidic pH, around 3.5 or so, in order to be really functional. When we were studying this process we looked and actually measured quantitatively exactly what the pH was and it did not get acidified so the pH, excuse me, I said it backwards, it did not get alkylated, the pH remains sufficient for the lysosomes to work but what happens is the lysosomes have problems bringing proteins such as cathepsins (which are some of the lysosomal enzymes that are used to break down proteins of different kinds). It had problems bringing them into the cell and allowing them to be processed properly. So the functioning of the lysosome is inhibited to some extent but, as Matt just explained, the most dramatic changes are in the abilities of lysosomes to depart, to undergo fission, so they form. They can't undergo fission, so they keep getting bigger and bigger by fusing with one another and yet the same process, the fusion process, which works with lysosomes fusing with other lysosomes but not being able to come apart again—in the case of lysosomes fusing with a vesicle called the autophagosome, which is the part of the process called autophagy. This prevents the actual fusion, so this drug, by inhibiting PIKFYVE, is having pleiotropic effects, i.e., multiple types of effects, on the cell and one could speculate that many of them haven't yet been identified. It's not a simple thing, where that enzyme has only one job, one function in one compartment. It actually blocks the concept of homogeneity of the lysosome.

MATTHEW: Homeostasis.

MDe: Thank you, when you get to be 78 years old, Matthew, it's dreadful how words do not pop into your head. In any event, there are at least three steps in lysosome homeostasis that can be interdicted—affected, inhibited, modified—by blocking this single enzyme, and we're in the process of learning more and more about how these inhibitors affect cellular metabolism.

GB: I think my next question is, can you describe more in detail your particular COVID research initiatives the methodology you all are using?

MDE: I'm not quite sure what exactly you want us to talk about?

GB: Yes, how you got started, the methods that you're using to do your research, equipment, techniques that you're using, any of that sort of thing where you are. You said right now you've done the cell cultures, but you hope to go to animal models, and I guess ultimately in people at some point.

MF: I'll start at the beginning. I'm trying to remember when we actually first connected last year. Early sometime last year, Mel reached out around March. Okay, my lab is a coronavirus lab. We worked on SARS-1 and MERS, that's what we do. It's studying pathogenesis and therapeutic development for those viruses. So as SARS-2 emerged, we were working up projects in the lab, really focused on a lot of antibodies and vaccines and drug screening, looking for inhibitors. Mel and Rup got in touch with me to ask if I would help test their family of inhibitors that they had been working on in the lab. We were kind of swamped at the time. I probably told them I was too busy. Luckily Mel was persistent and kept finding me and emailing. And so he brought up his inhibitors—he actually drove them to Baltimore. Right.

So we did test them at the time and the funny thing, if I remember from the beginning of the project, was that they kept killing the cells. We didn't realize why. It turned out that they were such potent inhibitors we could go down to much lower levels than what we thought we would need for regular drugs that we were studying for them to be effective. It was that we were basically a thousand-fold off of the range that we needed to use. He said he was telling me at the time, “No , it's too much.” I'm like, “Yeah, it doesn't make sense. Why do we have to go so low?” But eventually we got there and so through the year we've been working with them and really developing more and more of the assays in the lab. We have been looking at vero cells, which are a standard cell line in the lab which are African green monkey kidney cells, which we grow a lot of viruses in. We do a lot of studies in these cells and then transition over to human cell lines, human lung cell lines and primary cells as well, now going into mice,

And so over the period of time we've tested against SARS1 and done MERS and SARS-2 and then other seasonal corona viruses that we have in the lab, too. That just gives us common cold viruses like 229E and OC43 and so we've been studying those in cell culture. We've gone through looking and trying to figure out what the aspects of this is that are inhibiting, sharing data with Mel's lab as well as protein and lysates, so they can start understanding the biology behind what's going on as well from the virus side and the cell side. Now we're getting ready to go into mice where we can start doing these studies in animals to really understand how they are able to inhibit virus during infection in a mouse. At the beginning when we started talking to Mel, we didn't even have a mouse model. We didn't know what mice to use. We didn't know how to replicate or how to do pathogenesis studies for SARS-2. We've developed these things over the time as the general field has developed these over the year. So now we're going to mice with a mouse-adapted virus called ma10 which causes really nice weight loss and lung damage and replicates very well in the lungs of mice. We're using that for other drug studies as well. Then we're putting these new compounds into mice to see how well they protect, both when you treat before infection as well as after infection. Those are all things we're looking forward to doing.

GB: That's terrific. How long did each of these phases take? It sounds like it can be a while for each of them.

MDe: I have to tell you one marvelous anecdote that goes with Matt's beautifully abbreviated version. He has done an enormous amount of work and it's not easy to do. He makes it sound easy. How did this whole thing get started? Arup and I work in the National Institute of Child Health and Human Development. Arup is a tumor biologist, and my history has been DNA replication. I was hired for it. To make a long story short, one morning Arup came into the lab and said, “Did you read in the Washington Post about this Chinese report?”

AC: So I read about an article published in Nature during that time that I read about in the Post. A group somewhere in China is trying to test whether hydroxychloroquine would be effective in inhibiting infection. In our lab previously, postdocs tested that our PIKFYVE inhibitors, which also belong to the same kind of activities like chloroquine and hydroxychloroquine, but our PIKFYVE inhibitors are more potent than chloroquine and hydroxychloroquine and they're less toxic. So that prompted me to think about how to test our compounds to see whether we can inhibit this virus or not. So I started to learn who are the virologists/researchers in this area, or over the United States in general, working [on this]. I emailed many places but everybody either was very busy testing their own compounds or they didn't have any real particles. They were working with pseudo-particles which we really didn't want to test. Then one morning I came across an article in the Washington Post. There's a researcher at the University of Maryland, Matthew Frieman, who has the live virus in his lab. I immediately came back to the lab and wrote everything in the email and then immediately I received an email and showed it to Matt and that's how we got started.

MDe: Yeah, but to be fair, Gabrielle, Matt didn't have a chance because if I had not become a scientist, I would be selling used cars. So he got on the phone and we met Matt. We had never met each other but I had already tried to find somebody at the NIH and Matt said it perfectly: that every scientist is busy with their own projects, so when somebody walks in the door and says, “By the way, et cetera, et cetera, we'd like you to do something that might be important,” we are all reluctant to stop what we're doing or take someone's time to do that. But Matt and I and Arup had a wonderful conversation, and we went over and met, gave him the drugs. and, as Matt told you, he was very pleasantly surprised how potent these drugs were and in fact it's quite clear they do work. They really work in vitro quite well, something that's going to be nice, Matt, and I want to mention it to you, particularly in the mice.

Keep in mind these drugs are reversible. So Gabrielle, one of the problems in cancer research is that most of the drugs people use are very toxic to any cell that is proliferating, cancer or otherwise. That's why people's hair fall out, their gums start bleeding, all sorts of things, and literally most cancer research drugs are trying to kill the cancer before they kill the patient. These drugs are very reversible. They wash out within 24 hours and I suspect, Matt, you're going to have to inject the mice intraperitoneally, if that's the route you go, but you're gonna have to do it repeatedly, and when you stop doing it, the mouse will go on as a fairly normal animal. In contrast, the more toxic drugs, they're permanent. They induce DNA damage. The damage doesn't go away easily, so the mouse becomes permanently sick.

We hope that these drugs will help Matt and others put together a combination where you take a drug that targets, I was going to say autophagy, but let's just call it lysosome homeostasis—this general term—and by doing so, it enables other drugs which might be more specific for a virus or any other microorganism or a bacterial cell and allows the person in charge, the doctor doing this, to lower the levels of his toxic drug because it's now receiving help by making the cell much more sensitive to the problem. You try to find the center, it's called synergism. You try to find two drugs that work synergistically, having very different mechanisms of action, but when they're combined together, they give much more than an additive effect. Drug A does, say 100-fold and drug B does 50-fold and so forth, but instead of 150 you get a thousand-150. You've got a big jump in effectiveness. So I think this is going to be something very useful, Matt, in what you will do when you go to the mouse and you look at your mice. You're going to be able to put it in and be able to adjust it to lower levels. If you can think of other drugs that you might use in combination, that would be a great opportunity.

MF: The one thing we know about viral infections in general is that single drugs are difficult to treat these kind of infections. HIV uses a cocktail; hepatitis C is probably the only one I know of where an incredibly potent single drug, sofosbuvir, targets the polymerase. The idea is that if we have an effect with this drug, then we can layer it on and combine it with the other drugs we're finding that work as well and absolutely it also lowers the amount of drug you need for each one of them. So you lower toxicity, you lower off-target effects, and then you can get more doses out of the same amount of drug because you use less. All of this together is what you want to inhibit or at least slow down virus escape, because you're targeting either drug or the host of two different pathways.

MDe: Keep in mind these are medications which means that even if the viruses undergo mutation as they are doing in coronavirus now according to the popular press, it won't matter. The mutations may make the virus more effective, but these drugs are still going to block it because it doesn't care which virus is out there. If you have to come in by this avenue of endosomes, as an example, you're going to have a problem because the cells' mechanism that the virus needs is being screwed up. It's going to force a different generation. What do you think, Matt, a different generation of mutations or do you think the viruses would ever overcome such mutations?

MF: I think it's going to be harder for them to do it and even if they do evade these drugs in these combinations, then they're less fit generally. So as many times as they mutate, they lose a little bit sometimes with their fitness and ability to replicate. All of that is a good thing for us.

GB: It was very interesting. What have been some of the challenges that you all have experienced to date and similarly have you all had anything surprising that you have noticed so far in your research?

MF: The most surprising thing for me was how potent these drugs are, this family of drugs. You really can go down to levels that are a thousand-fold less than what our other drugs that work. I find that to be remarkable and I'm hoping this translates in vivo. But at least in vitro it works really well.

MDe: We remember we told you that on the phone and you just couldn't believe it? Arup, what do you have that's surprising?

AC: It was not surprising from the very beginning. I was very optimistic that these compounds were going to work because that paper, published in a very reputed journal, reported about the potency of chloroquine and hydroxychloroquine. We have in our lab already demonstrated these compounds are several folds more potent than chloroquine and hydroxychloroquine. From that point of view I was very optimistic that this confluence has worked at the in vitro level. We're hoping for the animal level, how it goes. It was not surprising to me.

In terms of challenges in COVID-19 research, I think last year at this time NIH was completely shut down. It was psychological challenges because I was the only person in this entire building working, not seeing anybody, so that kind of becomes scary that you're just working by yourself and also, I'm not expert in every single instrument or every single aspect of the assets, so a lot of researchers are not available here in the lab to help me or to discuss with them. From that point of view it was a little bit challenging, but it's getting better now. The maths lab has been extremely helpful moving this project forward. We are hopeful that we will have a very favorable outcome in this project.

GB: Did you and Dr. MDe work with coronaviruses before your lab? It seems like you all have worked a lot with cancer. What was the learning curve like changing from different diseases?

MDe: I've got the winning entry on this one. The most surprising thing was how much did my learning curve have to go up. In the year 2010, I published a whole book on DNA replication of all organisms on planet earth and how the whole thing comes together, et cetera, et cetera. We were not screening for drugs. We were not looking for anything to do with viruses or anything to do with the topographic. We were looking for small molecules that selectively inhibited a protein called geminin, which is essential in cancer cells to prevent the cancer cell from undergoing something called DNA re-replication, making mistakes let's say, and causing the cell to die. We thought if we had proven this is the case, the question was if we could get drugs that inhibited geminin, we would have a winner; we could target certain cancers.

The NIH gave me quite a bit of money to do the screening. We carried it out. It was very laborious. We did it in collaboration with NCATS and another institute which does these kinds of things. To make a long story short, we never found what we were looking for. Instead five molecules, a family of five molecules with very similar structure, showed up. The young postdoc here comes to this confession session—it's called confession sessions, Matt—so this young woman postdoc in the lab said, "I want to show you something. These molecules are all making the cells fill up with vacuoles. Look you can see them in the microscope." So I call this my mother's experiment because I could show it to my mother, God bless her, she's not here anymore, but show it to my mother and she would see these vacuoles as it's real easy. I said, "What do you think this means?" She says, "I think we're inducing autophagy." I said, "Really, what's autophagy?" I swear to God. So that's where I began.

We started off trying to look at mechanisms that regulate how eukaryotic cells reproduce their genome, DNA, and now we're out in the cytoplasm where I'm very unfamiliar. So it's been a big steep learning curve and it's a matter of trying to understand what turns out to be quite a complex system. All right, how's that for now?

Sounds good. It's interesting, but one of the things I love about this business is its serendipity. You can go on one of my site visits when I told him all about this stuff. He said, "Well, Mel, it sounds to me like you found lemon and you're making lemonade out of it." Meaning we didn't find what we were looking for, so we changed completely. What we were doing to match instead of trying to go back and find the inhibitors for the geminin. I've never gone back. I'm too old now. I don't have enough time left. So we went for something because it seemed so remarkably important.

What we had actually done without realizing it, we had set up a screen and said, ”Here are cancer cells and here are normal cells. I want molecules that selectively kill the cancer cells by increasing its DNA replication”, and it turns out when we put these inhibitors in, it happens. Not nearly as much as it would happen if I had an inhibitor against geminin, but it triggered the program that pulled these out. We started with something like 350,000 compounds that we screened, and we came up with five that actually worked together and then we discovered that two other labs that had published on something called vacuum, another one on pillow mold, which had come out a couple years earlier, and we realized why God, they are the same members of the family. It turns out that NCATS went back and looked in their library of 350,000 compounds and neither vacuum nor pillow mold are there or we would have found them. Isn't that interesting? That's funny, that's good.

So you know what it is, Gabrielle, you realize research is not a single directed arrow it's like a person walking through a forest trying to find their way and you have to pick a path to go. Sometimes you pick a good one, sometimes you're the bad one, so you have to back up to try another path. All of these things happen serendipitously. Matt was critical [to the research]. He was the only person we contacted or tried to contact that was willing to take a look at this stuff and, Matt, I am so pleased that it's worked out so marvelously for you.

GB: All right. Can you, as you've sort of started to speak about, can you each tell me a little bit more about what your roles have been with this research? It seems like you all have different areas of expertise that you are lending to this research and can you maybe talk a little bit about that?

MDe: You guys go first as I've been doing all the talking here.

AC: Okay, I'll start. As I was telling you earlier, it was just my idea initially that targeting PIKFYVE could be an effective way of inhibiting this virus. We have novel PIKFYVE inhibitors in the lab, we're working with a completely different aspect. In cancer biology that's the way the idea came, generated based on that background information. Then we came up with an assay that we're going to perform in collaboration with Matt's lab. As Mel was telling, we cannot work with the viruses on this campus at NIH so I was initially doing some of the assays where we tested PIKFYVE inhibitors on 06 cells and other human cells which we can infect with the virus because those cells express those receptors which are essential for the binding of the virus in order to get inside the cells. So initial in vitro assets without the virus were done here at Bethesda campus by me and then also at that time, when we were planning to test this compound, we already had planned to test these compounds in mice. Testing these compounds in mice is to see how much they can tolerate because we have no idea. These are novel compounds which no one has worked with in animals before. Also we don't know how much is too much [that would make] the mice to die, so I did those toxicity assays with these PIKFYVE inhibitors and came up with the information about how much these mice can tolerate, that will be effective maybe in inhibiting the viral infection but not producing any kind of visible side effects in mice. These are the assays that I have done here on the campus. Now Matt can talk about that.

MF: We are a coronavirus lab, so we were working in the BSL3 [Biosafety Level 3] with these viruses, both SARS, MERS, and SARS2. Our role in this was to do all of the live virus work of setting up the drug screens. We were working that in the lab when Mel contacted us and as we tried to optimize the screens and a variety of cell types to show how this virus replicated but also how the drugs were inhibiting this whole family of viruses. That was our side of this story.

GB: How many people work in your lab on this project?

MF: In my lab we have 10 people in total. Jimmy Logue, a grad student, has really been driving this project in all the cell inhibition work. He joined the lab in March of last year in the midst of the pandemic, so he has had the weirdest graduate student rotation in the history of rotations. But he's been driving a lot of the high throughput drug work that we're doing. He's done most of the cell culture work for this as well. Going into animals, I have grad students, including Rob Haupt and postdoc Robert Johnson, who are going to be working on all the drug work in there as well. That's exciting.

MDe: My personal interests actually are not involving coronaviruses. I'm going to retire at the end of 2022. I'll be 79 years old. I think that's reasonable. I love the kids. The reason I mentioned the key thing that fascinates me is tangentially related to the coronavirus and other types of problems and it's something Rup is involved in. You don't realize but Rup actually has two hands, and so one of his hands is in the coronavirus direction and the other hand has done some remarkable inventive analysis as to what types of cells are sensitive to these drugs. He's come up and, Rup, you can tell them more if you want to, but we have a manuscript that we're writing right now which we'll be submitting shortly in which we've identified specific types of cancer stem cells that are sensitive to this drug. That is great.

So the thing that fascinates me the most, and I'm going to try to complete before I retire, is understanding why some million cells are sensitive and others incredibly resistant. I love Matt's story of being introduced to the incredible discrepancy between what kills cells, what kills viruses. There is a range between what you need to stop a coronavirus from infecting the cells and what you need to kill a normal healthy fibroblast in a dish, so what I'm trying to understand is there are cancer cells which are autophagy dependent. These have been discovered by other people and remarkably they seem to be a minority at the moment. Even among melanoma, which is something another postdoc is working on, some of the melanoma cells are very sensitive while others are very resistant. The question we're trying to say is, “Okay, Why does this cell die so quickly while the other cells don't?”

We think we have an answer. We think we do understand the mechanism. If we're correct, that will tell us even more about how to use the drug and what kinds of cells to target, what you can do and what you can't do in the case of virus infections. For example, one of the problems Matt's going to run into is mice have about 100 different tissues, I think I've read something along that line. You can't go through them one at a time, but at some point, Arup is probably going to try. We're interested in saying, “Well, if you keep on putting more and more drug into a mouse, eventually it will start to die. What is it that's sensitive in the mouse, which tissue and the big problem?” If you ask any medical doctor about treating cancer patients, that is it. Well, mice can't tell you the problem, so they could be blind, and you won't notice. If they can have problems going on from the drug and you don't realize it and if we could identify which tissues are most sensitive, then that would help direct the kind of research.

Matt is going to say, “ Well, if this is going to be applicable to human beings” which is the obvious ultimate thing—oh by the way, I've already asked the NIH about phase one trials and they said we don't do them, so you have to get a company. They're very expensive, very elaborate these phase one trials. The tricky part is you need to get about seven or eight people willing to try a drug and they do it carefully and see whether or not they have side effects, ill effects that might inhibit.

GB: So you are using it against current, hopefully—your drug that you're creating—your inhibitors, are against coronaviruses, cancers. What other kinds of diseases do you think that it will prevent?

MDe: I was hoping it would prevent old age, but it didn't work well.

AC: Contributors have been implicated in autoimmune disease, Crohn's disease, so people have already tested these innovative pairs.

MDe: You can target anything that's involved with inflammation, originally targeting the arthritis things, like this Crohn's disease, it's an autoimmune disease. It creates feelings like arthritis, but the real problem again comes back to Matt's work. At some point every drug is toxic. There's just no way to escape that and you have to find out what you can or can't do. I'm encouraged. I don't worry too much about toxicity because cancer, they've done amazing things curing cancer and what they've used that was alluded to earlier, you use a cocktail of drugs, so you put them at lower concentration. You have multiple drugs, and you can end up literally killing the cancers before you kill the patient. Biggest problem, Matt, in the cancer business is that, if you don't get all the cells killed, they tend to come back, and like a virus you are selecting for the mutant one. I'm babbling. In the case of antibiotics, Gabrielle, the problem is, if we don't kill all the bacteria, the ones that survived are really good at resisting the drug you just used. So when it grows for your previous drug, like in tuberculosis, real problem, your previous drug is no longer good. Same problem in cancer research. Patients with recurring cancers are in serious trouble because it means the cocktail, the approach, radiation, whatever it was, you've only selected for those cells which are resistant, and they come back like gangbusters. So the trick is you have to get them all. Al Capone said that right before the Valentine's Day massacre. Have you guys ever heard of Al Capone?

GB: One of my last questions; this is a fun question. What are you each looking forward to doing when the pandemic is over?

MDe: Good question. Me ? I'm looking forward to going back to work. I want to go to the NIH. I'm stuck at home here. I'm serious. Hey, I'm playing the saxophone. That's what I'm doing. I gotta get to work.

MF: You gotta get vaccinated, Mel.

MDe: I've had my first one. I get my second one this coming Monday afternoon. Very exciting.

MF: What am I most excited for? I'm excited to go out to dinner again. I haven't been to a restaurant in 12 months, and I'd like to go back to our normal Saturday nights out.

MDe: Wait, Matt, aren't you married? Isn't your wife that remarkable chef cuisine? Don't answer that question. I just said that I want to work in the lab without wearing masks.

GB: Yeah, I can understand that. Well, is there anything else that the three of you would want to add as clinicians working on COVID research that you haven't mentioned yet?

AC: I recently have joined an NIH COVID-19 scientific interest group because eventually, I want to get this compound, if it works very well in the animal model, into phase-one clinical trials like Mel was mentioning. A clinician would be extremely helpful to initiate. None of us have an MD degree, so we need somebody with an MD degree or a physician or clinician. I started reaching out to some of the clinicians here to see whether they are interested if this compound shows remarkable sensitivity in the animal model. Would they be interested in taking the step forward or not? I have it in my mind. I've not just contacted them yet.

MDe: I'm rather reticent about it. Here's my opinion. I think physicians are trained to treat people. They're not trained in research, but I could pay for that. They rely way too heavily on vaccines, so when people mention viruses to physicians, the first thing they're going to do is to repeat what Matt warned us about. There are really very few drugs. It's very hard to target viruses with specific drugs. It's been tried quite a bit. So they rely on vaccines, flu for example. Everybody says, you get vaccinated, you don't have to think about it anymore. So at the moment I think physicians are counting on the idea that we'll have vaccines and this scourge called COVID-19 will simply disappear. Now, Matt, I don't know if that's going to happen. I keep reading about the new mutations and so forth, so if these viruses don't "disappear", then medications such as the ones we're talking about really become very important. You have this mindset—physicians love vaccines. Antibiotics were a big breakthrough because they work beautifully.

Vaccines were a big breakthrough because they work beautifully, but the two don't work against the same organisms. You can't use antibiotics against viruses and vaccines don't really treat bacterial diseases. To my knowledge there are vaccines against certain ones, but we'll see if they work. I've taken them. How about you, Matt, what do you think? Do you think physicians will be intrigued by your findings?

MF: I hope so. I think if we can show some animal studies that work, then that's certainly good. That's the next step. I don't think that this virus is going away anytime soon. I think it's looking like its going to be a seasonal coronavirus, so the goal would be to get it to flu-like levels in the winters. We'll see.

GB: Dr. Frieman, are you also working on other COVID-19 projects in your lab currently? Can you just maybe mention a couple of them?

MATT: Sure. I know Mel and Arup would wish that I was only working on theirs so that I could spend all my time on their drugs. We have a bunch of other things as we worked with Novavax on their vaccine. We did all their animal studies and a bunch of all their pre-clinical development on that vaccine. That was great. We worked with Regeneron on their monoclonals which are EUA approved. Also we did the animal studies there and the cell work and we worked with Astrazeneca on their monoclonals which are now in trials. Also, a bunch of drug studies looking at refurbished drugs and looking at other animal studies as well. You know there's just a whole lot of work to be done. It's been a very strange year. We have all kinds of new collaborators coming out of the woodwork and so luckily Mel and Arup have kind of risen to the top with a good drug.

GB: That's wonderful. Thank you all so much for talking to me and I hope that you have continued success. I look forward to keeping track of how your studies are going and I hope that you all continue to stay safe.

SCIENTISTS: Thank you, thank you.